Laboratory Examinations of Transmissible Spongiform Encephalopathies in Denmark during 2011

Jensen, Tim Kåre

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Report from the National Veterinary Institute, Technical University of Denmark
Bülowsvej 27, DK-1870 Frederiksberg C, Denmark

Introduction

The aim of this report is to give detailed information on the diagnostic examination on transmissible spongiform encephalopathies (TSE) performed in Denmark during 2011. The present annual report is the 16th on this topic published by the National Veterinary Institute, Technical University of Denmark (DTU-VET).

The report is made to fulfil the demands given by the EU Commission (Regulation No 999/2001 of the European Parliament and the Council of 22. May 2001) and the Office International des Epizooties (OIE) (Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th edition 2008, Chapter 2.4.6 and Chapter 2.7.13) regarding diagnostic examinations.

The DTU-VET is the national reference laboratory of bovine spongiform encephalopathy (BSE) and TSE/Scrapie, and therefore the results of all neuropathological examinations on BSE and Scrapie in Denmark are given in the present report as in previous years.

Assignments

This year's report includes all examinations of adult cattle, sheep, goats, pet animals, fur bearing animals, wild animals, and zoo mammals displaying behaviour disorders and/or neurological signs. The number of pet animals, wildlife, and zoo animals examined, however, do not necessarily include all examined animals in Denmark since neuropathological examination may have been performed at other laboratories without the knowledge of the DTU-VET. In no case, however, suspicion of TSE was made. Similar, neuropathological examination of adult ruminants without clinical signs or having diseases not compatible with BSE or Scrapie may have been performed at other institutions. However, the brainstems of these animals were examined according to the surveillance program described below.

The Danish BSE surveillance program included during 2011 the testing of the following bovine animals:

- Bovines with a clinical suspicion of BSE. All cases examined at DTU-VET
- Emergency slaughter bovines older than 48 months. Private, approved laboratories
- Bovines older than 48 months with remarks at the ante mortem inspection performed by the official veterinarian at the slaughterhouse. Private, approved laboratories
- All fallen stock of bovines older than 48 months. Private, approved laboratory and DTU-VET.
- All slaughter bovines older than 48 months, from July 1. 2011 all slaughter bovines older than 72 months, Private, approved laboratories.

The Danish TSE surveillance program included during 2011 the testing of the following small ruminants:

- Small ruminants with a clinical suspicion of TSE. All cases examined at DTU-VET
- All fallen stock older than 18 months. Private, approved laboratories.

DTU-VET carried all tests in agreement with Annex X in the Regulation (EC) No. 999/2001.

DTU-VET controlled test performance in the private laboratories. This included weekly submission of control data for review and an annual inspection visit. Proficiency trials of the private laboratories were from 2011 co-ordinated by the European Community Reference laboratory for TSE, AHVLA, Weybridge, UK

The laboratories' approval of daily set-up's are done according to specified criteria, incorporating results of positive and negative controls in IDEXX HerdCheck and PrioSTRIP assays. Assignment of test-positivity, test-negativity of tested tissue samples is made in relation to prefixed criteria as approved by the national reference laboratory. Retesting and possible reclassification of single samples with a positive or doubtful result takes place according to a prefixed schedule, in the case of borderline reactions or by demonstrable technical errors, such as grossly differing readings of duplicates or appearance of "clusters" of positive microtiter wells.

Staff

One veterinary pathologist trained in the diagnosis of TSE at the EU Community Reference laboratory for TSE, AHVLA, Weybridge, UK, have performed the neuropathological examinations of BSE or Scrapie at the Division of Veterinary Diagnostics and Research in Copenhagen. Two veterinary pathologists perform examination of pet animals, fur animals, wildlife, and zoo animals at the Division of Poultry, Fish, and Fur-bearing Animals in Aarhus. The pathologist at the Division of Veterinary Diagnostics and Research in Copenhagen do the microscopically, confirmatory examination of all suspected cases. In the Division of Veterinary Diagnostics and Research, another two veterinarians served the rapid tests/screening analyses whereas one senior scientist and one scientist performed the supervision of private, rapid testing laboratories. Furthermore, the TSE genotyping is performed by a molecular biologist.

Research

The principal goal is to develop or implement improved diagnostic means to ensure early and sensitive detection of TSE-agents in a rapid, high-throughput format. New developments in assay specificity will also be pursued, including the ability to discriminate between Scrapie agents and the BSE-agent. A range of synthetic, globular polymers (dendrimers) are being investigated for their ability to remove/unfold PrP- and other types of protein and peptide misfolded fibrils. These reagents may be combined with existing assays in order to improve prion strain differentiation.

The expression of PrP on sheep peripheral blood mononuclear cells and its link with the genetically determined susceptibility to scrapie has been investigated and has been submitted for publication The DTU-VET birth cohort model has been used to provide regular prognoses for BSE-cases in Danish-borne cattle forward to 2016.

Methods

Methodology for examination of clinically suspected cases of BSE or TSE/Scrapie

Clinically suspected cases of BSE or TSE/Scrapie were usually euthanized by intravenous injection of an overdose of barbiturate, and the heads were submitted - generally overnight - to DTU-VET. The whole brain was removed from the skull. A crosssectional sample of 5-10 gram from the brainstem just caudal to the obex region was taken and used for IDEXX HerdCheck according to the manufacture guidelines (the remaining material was kept at 5°C until the case was completed). The rest of the brainstem was fixed in 10% neutral buffered formalin for 3 days. Brainstem areas were selected according to the OIE Manual (medulla at the obex, medulla through the caudal cerebellar peduncles and midbrain through rostral colliculi), cut into 3-5 mm tissue blocks and post fixed for a few days with daily change of formalin. One half of the cerebrum was kept at 5°C until the case was completed. The rest of the brain material was fixed in 10% neutral buffered formalin for two weeks with change of fixative after one week. Transverse sections of the cerebrum and longitudinal sections of the cerebellum and the pituitary were, cut into 3-5 mm tissue blocks and post fixed for a few days with daily change of formalin. For histopathology, sections were stained with haematoxylin and eosin. Furthermore, immunohistochemistry (IHC) for demonstration of disease specific prion protein (PrP^D) was applied on the obex section of all cases.

If a case was inconclusive material was submitted to the EU Community Reference Laboratory for TSE, AHVLA, Weybridge, UK, for further examination.

In case of positive cases, fresh brainstem material was furthermore analysed by AFSSA Discriminatory Western Blotting for PrP classification.

Methodology for examination of animals-at-risk (fallen stock, etc.)

At DTU-VET the testing is performed according to the following scheme: The brainstem was removed on location (by the foramen magnum technique) and submitted to the DTU-VET in suitable containers (cooled or at room temperature and without any fixative). The first part of the testing was by IDEXX HerdCheck as the monitoring test, taking a 0.3 gram sample from one half of the obex region. The remaining brain stem with the intact other half of the obex region was stored refrigerated for confirmatory testing in case of a positive or inconclusive result. If IDEXX HerdCheck produced a negative result, the specimen was reported as negative without further examination. In case of a positive result, samples of the remaining brainstem were subjected to confirmatory testing by AFSSA Discriminatory Western Blotting for PrP classification, histopathology and IHC. The case was reported as BSE/Scrapie-positive if confirmatory testing revealed a positive result. In cases of severely autolysed / unsuitable material, that was IDEXX HerdCheck positive tissue was forwarded to AHVLA, Weybridge, for further examination.

Methodology for confirmatory examination of animals TSE positive by monitoring testing (when the rapid testing are positive)

In case of a positive or inconclusive result of the monitoring tests (rapid test) the confirmatory examinations were performed at DTU-VET according to the following procedure: A cross-sectional sample of 5-10 gram from the brainstem just caudal to the obex region was taken and used for the IDEXX HerdCheck analysis, as described above, if the monitoring test was performed at a private laboratory. In case of IDEXX Herd-Check positive cases a sample of brainstem was furthermore analysed in the AFSSA Discriminatory Western Blotting.

For additional confirmatory examination the brainstem was fixed in 10% neutral buffered formalin for 3 days. Brainstem areas were selected according to the OIE Manual (medulla at the obex (and medulla through the caudal cerebellar peduncles and midbrain through rostral colliculi if possible). Cut into 3-5 mm tissue blocks (obex), and post fixed for a few days with daily change of formalin. Sections were stained with haematoxylin and eosin as well as by immunohistochemistry (IHC) for demonstration of disease specific prion protein (PrP^D).

The case was reported as BSE/Scrapie-positive if confirmatory testing revealed a positive result. In cases of severely autolysed / unsuitable material that was negative in AFSSA Western Blotting tissue was forwarded to AHVLA, Weybridge, for further examination.

Methodology for examination of wildlife animals, pet animals, fur animals, and zoo animals

Mink older than seven months of age and other animals older than 1 year of age with clinical signs indicating a neurological disorder were examined for spongiform encephalopathies. Only mammalian species were included. The brain was divided into two equal parts by longitudinal section. One half of the brain was fixed in 10% neutral buffered formalin for two weeks. The fixative was changed after one week. The other half was stored at -18°C. The formalin fixed tissue was cut with 4 transversal sections into 5 equally large portions (ensuring that brain stem areas were selected according to the OIE Manual medulla at the obex, medulla through the caudal cerebellar peduncles and midbrain through rostral colliculi) and embedded in paraffin in a routine manner. Transverse sections of cerebellum were always included. Sections were stained with haematoxylin and eosin.

Results

BSE in bovines born in Denmark

During 2011 no case of indigenous BSE was diagnosed.

Scrapie in small ruminants born in Denmark

During 2011 five positive cases of indigenous TSE was diagnosed, all categorized as atypical scrapie according to AFSSA discriminatory WB testing and found in fallen stock animals.

Examination of suspected BSE and Scrapie cases

Through 2011, the head of five suspected BSE cases, and one suspected Scrapie case in a sheep were received for examination. The cause of submission according to order no. 1361, 19th December 2008, on Bovine Spongiform Encephalopathy and order no. 930, 9th September 2006 on Scrapie is given in Table 1.

The five BSE suspected cases were found negative in IDEXX HerdCheck and by IHC. Lesions consistent with listeriosis were found in three cases. Ependymoma was diagnosed in one case, while the last case showed caudal displacement of cerebellum with degeneration and necrosis of the caudal parts of cerebellum.. Lesions consistent with Scrapie were not observed in the sheep and it was PrP negative by IDEXX HerdCheck as well as PrP negative by IHC. An apparent cause of the neurological symptoms was obtained as lesions consistent with listeriosis were seen. Individual data on age and diagnosis is listed in Table 2. The monthly submission rates and the number of submission per regional veterinary office are given in Table 3 and 4.

Additionally, the laboratory received the whole carcase of a 14 years old pygmy goat (lab No. 2011-1727) from the Veterinary School with anamnesis of CNS symptoms and thus suspected of having scrapie. Region East was contacted and they decided that the case was not suspected for scrapie but should be examined as fallen stock. Histopathologically, lesions consistent with listeriosis were seen. The sheep was found negative in IDEXX HerdCheck.

Examination of animals-at-risk (fallen stock, etc.)

Approximately 22.000 bovines have been tested including 1099 cases analysed at DTUVET, Table 5. The remaining animals were analysed at private laboratories. No bo-vine animals-at-risk were found positive during 2011.

Moreover, approximately 8.000 sheep and goats have been tested, all analysed at private laboratories. Five sheep were found positive for atypical scrapie according to AFSSA testing (see table 6), the brain material of three cases were severely autolysed and not suited for IHC. Summary of the TSE positive cases during 2011, see Table 8.

Examination of healthy slaughter animals, confirmatory testing
The private, approved laboratories tested approximately 99.000 slaughter animals during 2011. The laboratories found 0 animals positive.

Examination of wildlife animals, pet animals, fur animals, and zoo animals
Lesions consistent with chronic wasting disease or other TSEs were not detected in wild fissipeds, and no TSE lesions were seen in other animals, Table 7.

Table 1. Cause of submission for suspected cases of bovine spongiform encephalopathy and Scrapie during 2011.
According to Order no. 800 of 13. July 2006 and 930 of 7 September 2006 on Bovine Spongiform Encephalopathy and Scrapie.

Cause	Numbers
Animals displaying behaviour disorders and/or neurological signs	6
Moribund animals without signs of infectious disease or traumatic signs	0
Other progressive diseases	0
No available information	0

Table 2. Summary of neuropathological findings in 5 cattle and 1 sheep suspected of having transmissible spongiform encephalopathy 2011.

Run. No.	Date	Lab. No.	HR.No.	CKR.No.	Breed	Age, years (months)	Region	Histopathology	Additional results regarding BSE	Year of confir-mation
1	16-03-2011	2011-10-655	53930	53930-2557	cow	4(51)	West	Lesions consistent with listeriosis	Negative by IHC and IDEXX HerdCheck	2011
2	16-03-2011	2011-10-656	53930	53930-2988	cow	2(29)	West	Lesions consistent with listeriosis	Negative by IHC and IDEXX HerdCheck	2011
3	22-03-2011	2011-10-692	118290	105705-00025	sheep	Unknown	East	Perivascular cuff-ing in cerebrum	Negative by IHC and IDEXX HerdCheck	2011
4	22-03-2011	2011-10-693	46923	469230-1227	cow	7(89)	West	Ependymoma	Negative by IHC and IDEXX HerdCheck	2011
5	02-05-2011	2011-10-1060	39719	39719-00051	cow	2(25)	West	Displacement and necrosis of cere-bellum	Negative by IHC and IDEXX HerdCheck	2011
6	14-12-2011	2011-10-2705	55799	54735-01214	cow	4(52)	West	Lesions consistent with listeriosis	Negative by IHC and IDEXX HerdCheck	2011

Table 3. Number of examined bovine spongiform encephalopathy and scrapie suspected cases per month by date of reception during 2011.

	Jan	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
BSE	0	0	3	0	1	0	0	0	0	0	0	1
Scrapie	0	0	1	0	0	0	0	0	0	0	0	0

Table 4. Number of examined BSE and scrapie suspected cases per regional veterinary office during 2011.

Regional veterinary offices	BSE	Scrapie
Region West	5	0
Region East	0	1

Table 5. Laboratory examinations at the DTU-VET 2011.
In accordance with the Danish surveillance programme and other tests.

Bovines. Group of animals	Samples	Negative	Positive
Emergency slaughter animals	0	0	0
Remarks at the ante mortem inspection	0	0	0
Fallen stock	1099	1099	0
Subtotal	1099	1099	0
Clinical suspects	5	5	0
Confirmatory testing of slaughter animals	0	0	0
Confirmatory testing of fallen stock	0	0	0
Total, bovines	1104	1104	0
Small ruminants. Group of animals			
Clinical suspects	1	1	0
Confirmatory testing of slaughter animals	0	0	0
Confirmatory testing of fallen stock	5	0	5
Fallen stock	2	2	0
Total, small ruminants	8	8	5
Wildlife animals etc.	6*	6	0

*Histopathology only.

Table 6. Results of confirmatory examination of fallen stock 2011(five sheep).

								Confirmatory examination		
Run No.	Lab. No.	Received date	CHR.No.	CKR.No.	Region	Priv.lab.	test	Histopath,	IHC for PrP	Additional results regarding BSE
1	2011-10-499	28-02-2011	43533	43533-02503	West	Eurofins	IDEXX Herd-Check, positive	Autolysis	Positive	Positive for PrP by IDEXX HerdCheck, atypical scrapie by AFSSA discriminatory WB
2	2011-10-1523	12-07-2011	27705	27705-0109	West	Eurofins	IDEXX Herd-Check, positive	Autolysis	Nd	Positive for PrP by IDEXX HerdCheck, atypical scrapie by AFSSA discriminatory WB
3	2011-10-1712	08-08-2011	92614	87743-00205	East	Eurofins	IDEXX Herd-Check, positive	Autolysis	Nd	Positive for PrP by IDEXX HerdCheck, atypical scrapie by AFSSA discriminatory WB
4	2011-10-2239	21-10-2011	113266	113266-0007	West	Eurofins	IDEXX Herd-Check, positive	Autolysis	Nd	Positive for PrP by IDEXX HerdCheck, atypical scrapie by AFSSA discriminatory WB
5	2011-10-2378	08-11-2011	64334	64334-0117	West	Eurofins	IDEXX Herd-Check, positive	Autolysis	Positive	Positive for PrP by IDEXX HerdCheck, atypical scrapie by AFSSA discriminatory WB

Table 7. Summary of neuropathological finding in wildlife animals, pet animals, fur animals, and zoo animals necropsied due to behaviour disorders and/or neurological signs during 2011. Only mammalian species have been examined.

Lab. No.	Species	Diagnosis	Age
2011-52-367	Roe deer (*Capreolus capreolus*)	Histopathological changes characteristic of neuron vacuoles in cerebrum and focal loss of Purkinje cells in cerebellum	adult
2011-52-742	Dog (Canis *familiaris*)	hepatic spongiform encephalopathy	adult
2011-52-847	Dog (Canis *familiaris*)	Suppuratiev meningoencephalitis	adult
2011-52-1136	Dog (Canis *familiaris*)	Multifocal , suppurative meningoencephalitis	adult

In addition, 2 roe deer (Capreolus capreolous) without behavioral or neurological signs were investigated for histopathological changes of spongiform lesions with negative results.

Table 8. Summary of the five indigenous TSE cases in sheep in 2011.

Lab. No	Received date	Group	Breed	Age	Priv.lab.	IHC for PrP	Additional results regarding TSE	TSE genotype	Conclusion
2011-10-499	28-02-2011	Fallen stock	sheep	15	Eurofins, IDEXX HerdCheck, positiv	Cerebellum << obex.	Positive for PrP by IDEXX HerdCheck, atypical scrapie by AFSSA discriminatory WB	AHQ/AHQ	Atypical scrapie, no signs of BSE.
2011-10-1523	12-07-2011	Fallen stock	sheep	5	Eurofins, IDEXX HerdCheck, positive	nd	Positive for PrP by IDEXX HerdCheck, atypical scrapie by AFSSA discriminatory WB	AFRQ/AFRQ	Atypical scrapie, no signs of BSE.
2011-10-1712	08-08-2011	Fallen stock	sheep	>13	Eurofins, IDEXX HerdCheck, positiv	nd	Positive for PrP by IDEXX HerdCheck, atypical scrapie by AFSSA discriminatory WB	AHQ/ARQ	Atypical scrapie, no signs of BSE.
2011-10-2239	21-10-2011	Fallen stock	sheep	9	Eurofins, IDEXX HerdCheck, positiv	nd	Positive for PrP by IDEXX HerdCheck, atypical scrapie by AFSSA discriminatory WB	Not possible*	Atypical scrapie, no signs of BSE.
2011-10-2378	08-11-2011	Fallen stock	sheep	8	Eurofins, IDEXX HerdCheck, positiv	Cerebellum >>> obex	Positive for PrP by IDEXX HerdCheck, atypical scrapie by AFSSA discriminatory WB	AHQ/AHQ	Atypical scrapie, no signs of BSE.

*Due to DNA amplification inhibitors in the sample.